FUNCTION OF MOLLUSCAN STATOCYSTS

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ABSTRACT. The gravity sensors of most molluscs are spherical organs called statocysts. The wall of the sphere contains mechanosensory cells whose sensory cilia project into the lumen of the cyst. The lumen is filled with fluid and dense "stones", the statoconia or statoliths, which sink under the influence of gravity to load and stimulate receptor cells which are at the bottom. The composition of the statoconia is known in only a few species. Data presented here suggest that they are aragonite in *Aplysia*. The statoconia of *Aplysia californica* are shown to be calcified about a lamellar arrangement of membranes. Similar lamellar membrane arrangements are seen within the receptor cells, and their possible role in the formation of the statoconia is discussed. Scanning electron micrographs of unfixed statoconia reveal plate-like crystallization on their surface. Elemental analysis shows a relatively high strontium content. This is of interest in light of the recent report (Bidwell *et al.*, 1986) that strontium is required in the culture medium of several laboratory-reared molluscs before statoconia develop.

INTRODUCTION

In all molluscs studied to date, gravity reception is mediated by bilateral paired statocysts. The general form of the statocysts is that of a fluid-filled sac with ciliated mechanoreceptor cells along its wall. The ciliated surface of the receptor cells faces the lumen of the cyst. The gravitational stimulus is transduced by the interaction of stones, generally of calcium carbonate, in the lumen with the cilia of the receptor cells. Since the stones have a greater specific gravity than that of the fluid, gravitational forces are normally exerted on the receptor-cell cilia. In some species, either a single stone or a concretion of many smaller stones exists, in which case the mass is referred to as a *statolith*. In other species, many individual stones move independently under the influence of gravity, animal movement and beating of the sensory cilia; in these cases, the stones are referred to as *statoconia. In those statoliths made up of multiple adherent stones, the individual stones are also referred to as statoconia. Thus, the term statoconia is usually used to denote relatively small (1–50 μm* diameter), independent, paracrystalline elements. In this paper we will consider the interactions of the statoconia with the sensory cilia in the *Aplysia* statocyst, which lead to sensory transduction and the detection of gravity. Different suggested sites and mechanisms of generation of these stones, will also be considered.

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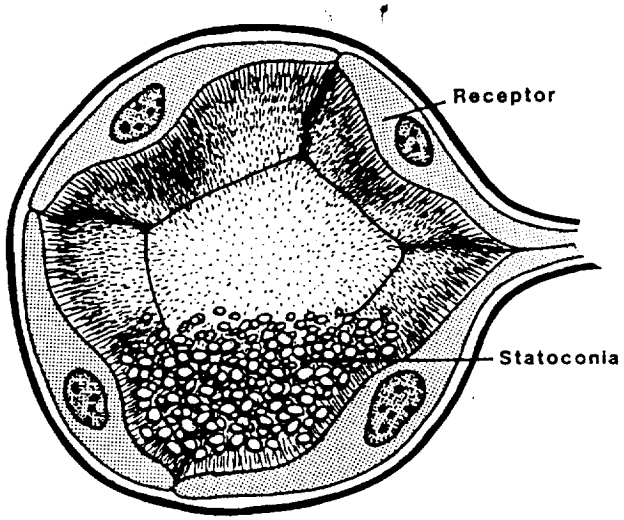

FIGURE 1. Schematic drawing of *Aplysia californica* statocyst. Note that supporting cells between receptor cells are not indicated. Modified from Gallin & Wiederhold (1977).

MATERIALS AND METHODS

Specimens of *Aplysia californica* were obtained from Pacific Bio-marine Laboratories, Venice, California. Animals were maintained in the laboratory in artificial sea water (Instant Ocean™) which lacks strontium (Bidwell *et al.*, 1986). Specimens were 20–125 grams. For light and transmission electron microscopy (TEM), statocysts were dissected free from live circumesophageal rings of ganglia and immersed directly into fixative. The material for Figures 2, 3 and 4 was fixed for 4 h with 3% glutaraldehyde in 0.15 M sodium cacodylate buffer solution, prepared with artificial sea water (Instant Ocean™) (1,004 mOsm, pH 7.35). Specimens were post-fixed in 1% osmium tetroxide for 1 h, dehydrated in a graded series of ethanols and embedded in epoxy resin.

Sections (1 μm) were retained at 10–20 μm intervals through the cyst and stained with 0.5% toluidine blue in 1.0% sodium borate for examination by light microscopy. For TEM, 90 nm sections were cut and stained with uranyl acetate and Reynold's lead citrate. Thin sections were examined in a Philips 301 TEM. The statoconium in Figure 7 was decalcified prior to dehydration by immersion in cacodylate buffer with the pH adjusted to 5.3 for 24 h. For the thin section in Figure 6, fixation was in 2.5% glutaraldehyde in 250 mM of 2,4,6-trimethylpyridine (s-collidine) buffer (pH 7.4) for 12–24 h. No additional decalcification was performed. For scanning electron microscopy (SEM) of isolated statoconia (Figures 8) the stones were isolated into deionized water which was blotted away, rinsed and blotted once more, on carbon planchets. The mounted specimens were coated with 60/40 gold/palladium at 20 nm thickness and examined in a JEOL JSM 35 SEM at 20 kV. For elemental analysis, similarly prepared but uncoated statoconia were examined in the same electron microscope using a Tracor Northern energy dispersive X-ray detector and a Tracor Northern NS880 X-ray analysis system.

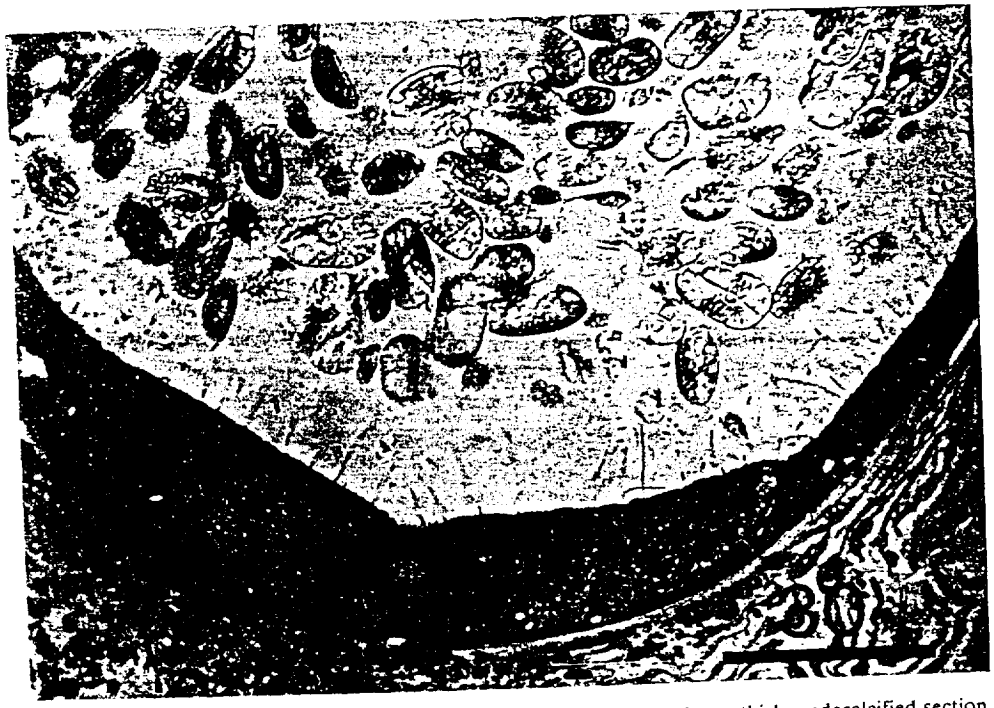

FIGURE 2. Light micrograph of lower portion of an *Aplysia* statocyst. 1 μm thick, undecalcified section, stained with toluidine blue. Bar = 30 μm. Mod. from Wiederhold *et al.* (1986).

For physiological recording, the circumesophageal ring of ganglia was removed and mounted in an artificial-seawater bath maintained at 15° C. Part of the pedal ganglion was removed to expose the statocyst and individual receptor cells were impaled with glass microelectrodes having tip resistances of 50–100 megOhms. A plexiglass dish holding the preparation and micromanipulators holding the microelectrodes were mounted on a tilting table which could be rotated through 40° in either direction from horizontal.

STRUCTURE OF STATOCYSTS

The general plan of the *Aplysia* statocyst is representative of the simplest form of molluscan gravity receptor (Coggeshall, 1969; McKee & Wiederhold, 1974). The statocysts are paired, one being located between the pedal and pleural ganglia (parts of the circumesophageal ring of ganglia) on each side of the animal. Figure 1 is a schematic drawing of the organ. Each statocyst is a sphere, approximately 200 μm in diameter. The wall of the cyst is made up of a basal lamina surrounding 13 large receptor cells. Each receptor cell has its own axon and these 13 axons join to form the statocyst nerve, indicated schematically in Figure 1 as proceeding to the right of the cyst. There is also a larger number of small supporting cells peripheral to and interposed between the receptor cells, which are not indicated in this figure. The luminal surface of the receptors is covered with many (approximately 700 in several examples counted) long cilia (approximately 12 μm long, as illustrated in Fig. 2, below). The statoconia are indicated

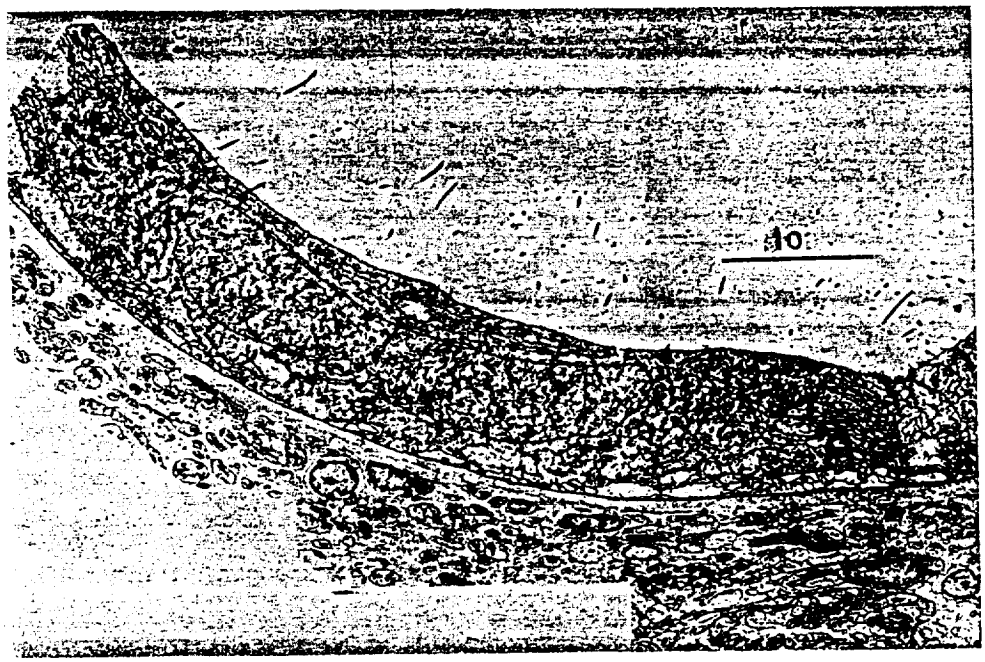

FIGURE 3. Montage TEM of section through one receptor cell. Note large nucleus, intracellular organelles and many cilia. Bar = 10 μm.

schematically as ellipsoids. They vary from approximately 3 to 20 μm in diameter. The number of statoconia varies from only one in larval *Aplysia* (Bidwell *et al.*, 1986, Gallager *et al.*, 1988) to as many as 1,000 in an adult (McKee & Wiederhold, 1974). The cilia all have the typical morphology of motile cilia, possessing a central pair of microtubules as well as 9 doublet microtubules around the periphery of the cilium. In fact, these cilia are motile, as well as sensory, as will be discussed below. Variations in the form of statocysts in other molluscan species are described in Wiederhold, *et al.*, 1986.

A more accurate picture of the *Aplysia* statocyst is given in Figure 2, a cross-section, illustrating statoconia and receptor cells at the "bottom" of the cyst. Sections of many statoconia are seen, as well as cilia on the luminal surface of three receptor cells. The statoconia are ellipsoidal in shape, varying here from 5 × 2 μm to 17.5 × 5 μm. Note several cilia whose complete length of 12 μm can be seen. In live preparations, the mass of stones can be seen to fill the bottom one – third of the lumen. Note that the statoconia appear to be free from one another. If a dissected live *Aplysia* statocyst is rotated under a microscope, the statoconia can be seen to tumble over one another as they fall to the new "bottom" of the lumen. Figure 3 is a montage illustrating a cross section of an entire receptor cell. Note the large nucleus and many intracellular organelles, as well as the large number of cilia cut in cross-section. Figures 2 and 3 illustrate that individual receptor cells can be quite large, compared to mammalian hair cells. The *Aplysia* receptor cells are typically rounded plates, 10 to 15 μm thick and up to 100 μm on a side. In the example shown in Figure 3, the luminal extent of the cross section is 80 μm from

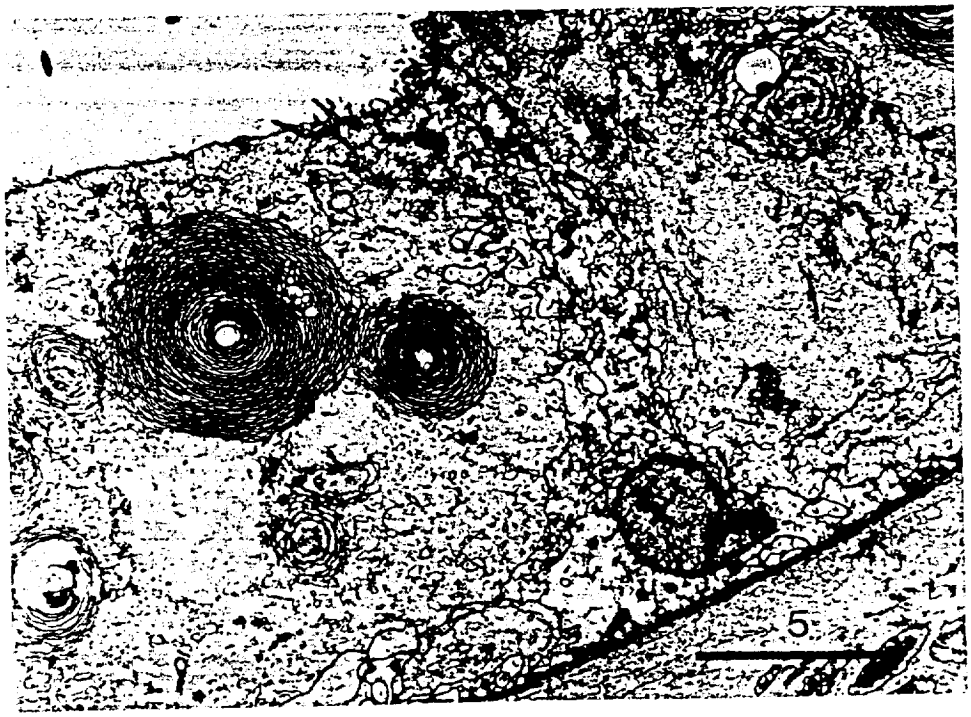

FIGURE 4. TEM of two statocyst receptor cells with several supporting cell processes interposed between them. Note prominent lamellar bodies in both receptor cells. Bar = 5 μm. Mod. from Wiederhold *et al.* (1986).

one end of the receptor cell to the other. From the size of the statoconia, relative to the spacing between cilia, it is apparent that when a single statoconium strikes the cell surface, it will interact with several cilia nearly simultaneously.

Figure 4 shows the border region between two receptor cells, with several small supporting-cell processes separating them at the luminal surface. Several characteristic organelles can be seen in this figure. Smooth endoplasmic reticulum is seen in the receptor cell, approaching its border with the supporting cell. Multiple lamellar bodies are frequently seen in this position, near the junction with the supporting cells. Here, 6 lamellar bodies are seen in the left-hand receptor cell and two in the right-hand cell. The large lamellar body in the left-hand cell is 6.3 μm in diameter.

FUNCTION OF STATOCYSTS

Molluscan statocysts have provided useful preparations for the study of mechanoelectric transduction in ciliated mechanoreceptor cells. Early studies concentrated on response properties of the statocyst nerve fibers to tilt and rotation (*e.g.*, Wolff, 1973). The large size of the receptor cells in *Aplysia californica* made them attractive for intracellular recording. In favorable cases, intracellular recording with a micro-electrode has been maintained for up to 8 hours (Gallin & Wiederhold, 1977; Wiederhold, 1974, 1977, 1978).

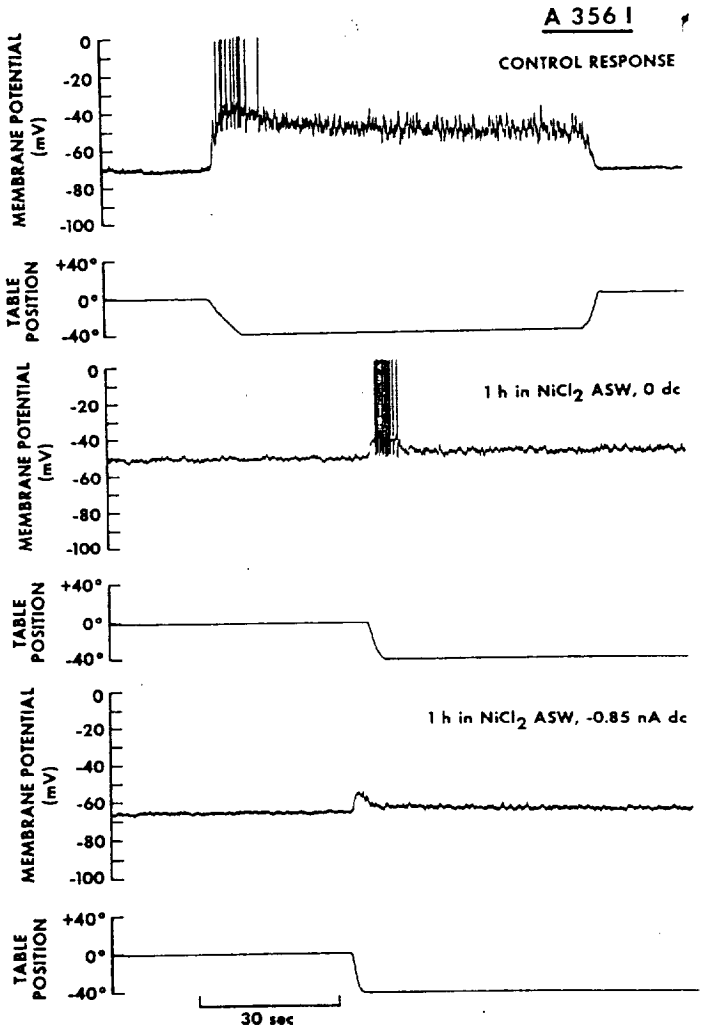

FIGURE 5. Intracellular responses of a statocyst receptor cell to downward tilt through 40°. Top pair of traces with preparation in normal sea water; middle pair, 1 h after being in sea water with 10 mM Ni^{2+} added; bottom pair, in 10 mM Ni-sea water with membrane potential restored to -70 mV when table is in horizontal position. In each pair, upper trace is membrane potential and lower trace is tilting table position.

Aplysia statocyst receptor cells typically have resting membrane potentials ranging from -50 to -80 mV, input resistance of the order of 100 megOhm and electrical time constants near 100 msec. The large input resistance and long time constant facilitate the measurement of membrane resistance using a single intracellular electrode and a bridge circuit (Gallin & Wiederhold, 1977). We have recorded from receptor cells in a

preparation of the circume-sophageal ring of ganglia, including the statocysts, mounted in a small chamber containing cooled artificial sea water, on a tilting table. When the table is tilted from a position in which the recorded cell is above the level of the statoconia to a position where the recorded cell's cilia are in contact with the stones, large depolarizations and generation of action potentials are observed. In the example illustrated in the upper two panels of Figure 5, the receptor cell has a resting potential of -70 mV with the table in the horizontal position (table position of 0 degrees). When the table is tilted to bring the recorded cell down (-40 degrees), the cell depolarizes by 30 mV and a series of action potentials lasting approximately 10 sec is generated. The membrane potential then partially repolarizes, so that the action potentials cease, but the increase in potential fluctuations continues throughout the cell-down tilt.

The action potentials carried in the statocyst nerve convey orientation information to the cerebral ganglia. Only those cells at the bottom of the cyst are activated, giving the central nervous system information concerning which direction is "down". The depolarization in response to this physiologic stimulus can be as large as 50 mV. By passing small current pulses through the recording electrode and across the receptor cell membrane, it is also possible to measure changes in membrane conductance associated with the response. Although the electrical characteristics of the receptor-cell membrane are very non-linear, which can complicate the analysis (Wiederhold, 1977), it can be shown that the depolarization is due to an increase in membrane conductance caused by the mechanical stimulus. By changing the ionic composition of the artificial sea water bathing the preparation, it has been shown that the conductance increase caused by stimulation results primarily from an increase in conductance to sodium, presumably either on the ciliary membrane or the luminal surface membrane of the receptor cell (Gallin & Wiederhold, 1977). By eliminating the sodium in the bathing medium, responses to tilting are eliminated within 15 to 20 min, suggesting a relatively unrestrained exchange between the cyst lumen and the external medium. This is consistent with the finding that a microelectrode in the lumen records no standing potential and virtually no electrical resistance, relative to a reference electrode in the bath. Thus, ions and trace elements in the extracellular spaces of the animal will have access to the cyst lumen and the luminal surfaces of the receptor and supporting cells.

An interesting feature of the physiological responses of statocyst receptor cells is the large increase in fluctuations in membrane potential associated with the depolarizing response to tilt or rotation, as seen in the top panel of Figure 5 (see also: Gallin & Wiederhold, 1977; Wiederhold, 1978; Grossman *et al.*, 1979). When an *Aplysia* statocyst preparation is viewed under a dissecting microscope, the statoconia can be seen to be in continual, random movement. This has also been noted in *Clione limacina* (Tsirulis, 1974), *Pecten maximus* larvae (Cragg & Nott, 1977) and *Hermissenda crassicornis* (Grossman *et al.*, 1979). Direct observation of active ciliary beating is reported in the statocysts of *Helix* (Laverack, 1968), *Lymnaea stagnalis* (Geuze, 1968) and *Hermissenda* (Stommel *et al.*, 1980). When nickel chloride ($NiCl_2$) (10 mM) or serum from patients with cystic fibrosis (both of which can block ciliary motility--see Lindemann, *et al.*, 1980; Danes & Bearn, 1972), were added to the sea water bathing an *Aplysia* statocyst, the random motion of statoconia ceased (Wiederhold, 1978). The motion is thus, in all

likelihood, imparted by the active beating of the receptor-cell cilia. Application of nickel or cystic fibrosis serum also greatly reduces the voltage fluctuations and the magnitude of the depolarizing response to tilt. In the middle two panels of Figure 5, a -40° tilt was applied 1 h after adding 10 mM $NiCl_2$ to the artificial sea water bathing the preparation. In this particular case, the cell had depolarized by 20 mV while the preparation was in the Ni enriched sea water although, averaging across all preparations, there was no consistent effect of Ni on either the resting membrane potential, the cell's input resistance or the threshold or amplitude of action potentials. In the Ni sea water, the depolarization caused by the same tilt as in the top trace was only 10 mV and the train of action potentials lasted only 5 sec. If the membrane potential was restored to -70 mV with the table horizontal (bottom two panels), by passing -0.85 nA current through the microelectrode, the depolarizing response lasted only approximately 5 sec, no action potentials were generated, and the potential fluctuations barely increased during the maintained tilt. The fact that the depolarizing response of the receptors is greatly reduced by the same treatments that block active ciliary beating, led Wiederhold (1976, 1978) to conclude that the actual transduction mechanism involves the cilia actively striking the statoconia during their ongoing beating, rather than the statoconia passively deflecting the cilia, as is thought to be the case in vertebrate hair cells. Stommel *et al.* (1980) have come to similar conclusions from experiments in which ciliostatic agents were injected into *Hermissenda* statocyst receptor cells.

From this synopsis, it can be seen that the physiology and biophysics of sensory transduction in molluscan statocysts is understood in reasonable detail. The manifestation of collisions between receptor-cell cilia and individual statoconia, in the prominent voltage fluctuations associated with the responses, suggests that the physical and structural characteristics of the statoconia could appreciably affect the physiologic responses of these gravity receptors. These aspects will be treated in more detail in the next section.

FORMATION AND COMPOSITION OF STATOCONIA

Most molluscan statoconia described to-date have forms similar to that illustrated in Figures 1 and 2. The major exceptions are some of the bivalves, which have large "single-crystal" statoliths or statoliths which are concretions of statoconia (Morton, 1985), and the dibranchian cephalopods (squid, cuttlefish and octopus) in which the normal statolith has a complicated shape and is made up of a concretion of small spindle-shaped crystals (Dilly, 1976; Colmers *et al.*, 1984). The finding that in the same species, *Aplysia californica*, there is only a single statolith in larval animals (Bidwell *et al.*, 1986, Gallager *et al.*, 1988) and approximately 1,000 statoconia in adults (McKee & Wiederhold, 1974) indicates that stones are added to the cyst lumen during development. The statocysts are one of the first components of the *Aplysia* nervous system to develop. Fully formed statocysts are present in larval animals when they hatch, 10 days after fertilization. At this stage the only other components of the nervous system which can be identified are the cerebral and pedal ganglia (Kriegstein, 1977a,b). Bidwell *et al.* (1986) note the presence of a statolith in normal 5 day embryos. Coggeshall (1968) emphasizes that the diameter of the statocyst varies little between the smallest (1 g) and the largest animals studied (300 g) and that the number of receptor cells (13) is constant.

It is not known when in the development of these animals the number of statoconia increases or whether this is an ongoing process throughout their life.

There are several suggestions in the literature concerning the site of generation of statoconia. Laverack (1968) was one of the first to apply EM techniques to the investigation of molluscan statocysts. He termed the large ciliated cells, of which there are 13 also in *Helix*, "giant" cells, but concluded that these could not be receptor cells, largely because all sensory receptors described to that point were small columnar cells. Thus, Laverack concluded that the supporting cells were the receptors. However, he did illustrate "fully formed" statoconia, approximately 7 μm along their major axis, within a giant cell. He states that "the bulk of the calcareous material leads to herniation of the capsule...when the cell contains a number of statoliths they are released into the lumen of the statocyst, by disruption of the cell." Laverack also notes striking lamellar bodies, similar to those illustrated in Figure 4, in the giant (receptor) cells, near intracellular statoconia, but concludes that the lamellar bodies do not seem to be related structurally to the statoconia. He states that in regions of the giant cell where lamellar bodies are numerous, the cell frequently becomes detached from the capsule and "disintegrates into the lumen of the statocyst". We have occasionally seen such disruption with impressive amounts of regular, but more loosely arranged membranes than those illustrated in Figure 4, within the lumen. However, we could not be assured that this was not a form of degeneration, perhaps due to trauma associated with either dissection or preparation of the specimen.

In contrast to Laverack's suggestion that the statoconia are generated within what are now known to be the sensory receptor cells (Laverack, 1968), several investigators have illustrated small statoconia in invaginations of the luminal surface of supporting cells, between receptor cells and near the region where the nerve leaves the cyst. Tsirulis (1974) illustrates 5 statoconia, up to 12.5 μm along their major axis, within one supporting cell and another, 4 μm long, apparently emerging from a supporting cell in *Clione limacina*. Geuze (1968) presented evidence that in both normal and regenerating statocysts of *Lymnaea stagnalis*, the statoconia are produced by supporting cells. After puncturing the statocyst and removing both the statolymph and statoconia, the statocysts completely returned to normal appearance in 48 h. At 12 h after puncture, statoconia of low electron density were seen within vacuoles at the apical surface of poorly differentiated cells. In "subadult" normal animals, examples are shown of statoconia in broken vacuoles, in the cyst lumen, adjacent to the apical surface of supporting cells. Kuzirian *et al.* (1981) also demonstrated what is described as a forming statoconium within a supporting cell in *Hermissenda crassicornis*. However, it is difficult to distinguish this from a portion of a lamellar body. The example illustrated is near the junction between supporting and receptor cells, where processes of the two frequently interdigitate, and it is difficult to distinguish one cell type from the other. Cragg & Nott (1977) described one cell in each statocyst of the pediveliger *Pecten maximus* which is comparable in size to the ciliated receptor cells, but contains no cilia itself. This cell is said to contain inclusions resembling the variety of statoconia they describe in the cyst lumen and they suggest that this cell generates these stones and expels them into the cyst lumen. No one else has described such a cell. To date, we have not identified indisputable statoconia within either receptor or supporting cells in *Aplysia californica*.

FIGURE 6. TEM of several statoconia which were not decalcified beyond glutaraldehyde fixation in s-collidine buffer. Note concentric membranous ring structure in statoconia. Bar=5 μm. Mod. from Wiederhold, *et al.* (1986.

Of course, all of these studies suggesting that the statoconia are generated within either the receptor or supporting cells are based on static anatomical methods and the possibility that the stones penetrated into the cells from the lumen, possibly as a post-mortem artifact, cannot be excluded. In fetal rat, Salamat *et al.* (1980) demonstrated that developing otoconia are released from vesicles on the surface of cells in the sensory epithelium of the sacculus.

It is clear that the mineralization of molluscan statoconia is laid down on a biological membrane structure. Vinnikov *et al.* (1980) generalized that the "otoliths" of the "lower" invertebrates, the coelenterates and ctenophores, are formed by an "endogenic" intracellular mode, while those of the molluscs and arthropods are formed by an endogenous deposition upon an extracellular structure. Figures 6 and 7 illustrate statoconia within the lumen of *Aplysia californica* statocysts prepared and partially decalcified by different methods. Similar figures have been shown by Coggeshall (1969) for *Aplysia* and Kuzirian *et al.*, (1981) for *Hermissenda crassicornis*. Concentric membranous rings can be seen in most of the stones in Figure 6 and in the left-hand stone of Figure 7, but not in the other statoconium in Figure 7. The lack of a visible ring structure is not uncommon. Presumably the whole stone is made up of concentric ellipsoids and if such a structure were cut near an end, the ring structure could be missed, as in cutting a section from the side of an onion, rather than cutting across its center. It seems likely that the statoconia grow after their initial formation by adding successive layers. If this is the case, the lamellar bodies (Fig. 4) could provide the membrane for such deposition. As noted above, Laverack (1968) described "disintegration" of the receptor cells, in regions with numerous lamellar bodies, into the cyst lumen, a phenomenon which we have observed in *Aplysia* statocyst cells of uncertain condition. Williams (1977) described a process by which lamellar bodies, which appear similar to those in Figure 4, in the alveoli of fetal rat lung, exocytose at the cell surface to produce the tubular myelin which is thought to serve as a store of phospholipids for pulmonary surfactant. Thus, there is a precedent for similar structures being exocytosed to provide extracellular membrane. In Figures 6 and 7, the outer membrane layer appears more irregular than do the inner rings. Perhaps as the outer layer calcifies, the membrane is packed into a more confined space. Vinnikov *et al.* (1980) illustrated exceptionally thick

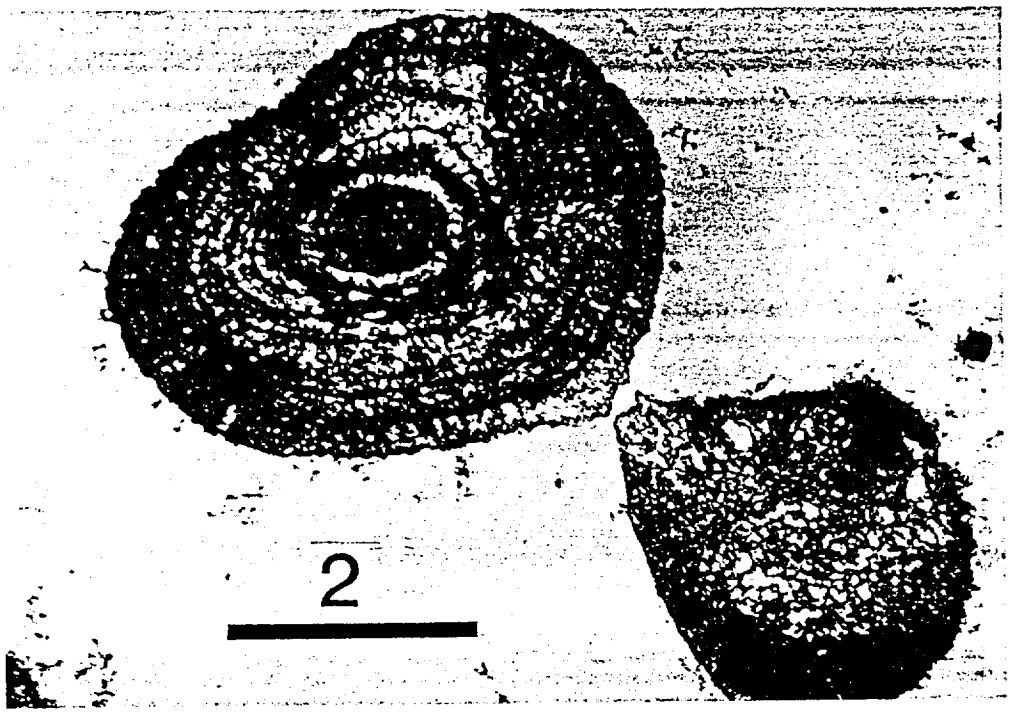

FIGURE 7. TEM of two statoconia which were decalcified in pH 5.3 buffer for 24 h. Note that ring structure is prominent in left stone but not in right. Bar = 2 μ m. Modified from Wiederhold, *et al.* (1986).

and electron-dense outer membranes in thin sections of statoconia from the limpet *Acmaea pallida*.

All of the statoconia described above have gone through some form of fixation, which could alter the elemental composition and surface structure. Ballarino (1985) has shown that even deionized water can etch avian otoconia. To minimize such changes, we have examined statoconia from one preparation in which the statocysts were dissected free from a live specimen (*Aplysia californica*) and bisected in a drop (at least 1,000 times the volume of the statocyst) of deionized water, directly on a carbon planchet. The water was blotted away and another drop placed and blotted to rinse the stones a second time. An SEM of a statoconium prepared in this manner is shown in Figure 8. The surface of this stone is more planar and angular than those previously illustrated in the literature (*e.g.*, McKee & Wiederhold, 1974; Kuzirian *et al.*, 1981). The stones in Figure 8 appear to have much sharper corners than the fixed stones. The unfixed stones show plate-like irregularities on their surface which appear to have cleaved at a preferred angle, suggesting a single-crystal formation.

To investigate the elemental composition of the statoconia, uncoated preparations similar to those shown in Figure 8 were studied by energy-dispersive X-ray microprobe analysis. Figure 9 is an X-ray energy spectrum obtained from a whole statoconium.

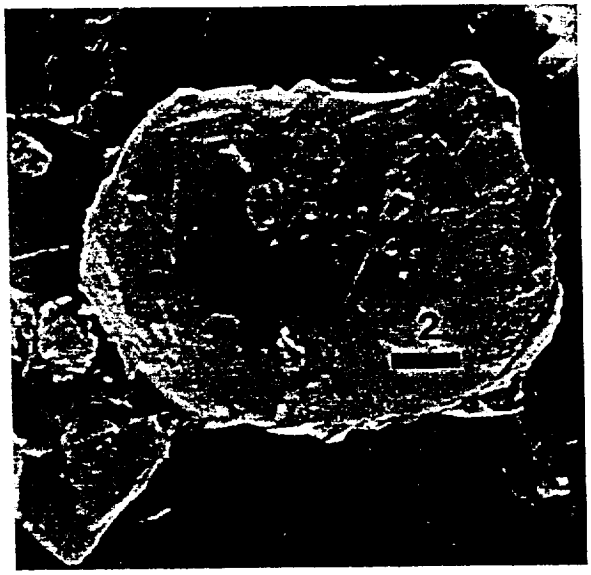

FIGURE 8. SEM of a large (14 μm dia.) statoconium prepared by vital dissection into deionized water. Irregular background is unpolished surface of the carbon planchet. Gold-palladium coating. Bar=2μm. Mod. from Wiederhold *et al.* (1986).

Note the prominent CaKα peak (which goes off scale in Fig. 9) as would be expected for $CaCO_3$. Small but significant amounts of sodium, magnesium, strontium, sulfur and chlorine are also present. The aluminum peak is an artifact from the X-ray detector housing. Note that no phosphorus peak is seen, indicating that there is probably no hydroxyapatite in the statoconia. In an attempt to quantitate the relative molar amounts of calcium and strontium present in the statoconia, a deconvolution algorithm was used to determine the contributions of these two elements to the spectra. To calibrate these measurements, standards of pure calcium fluoride (CaF_2) and pure strontium fluoride (SrF_2) were analyzed. The average Ca/Sr molar ratio derived from 6 statoconia was 97:1 $\pm$ 3 (mean $\pm$ std. error). The average number of counts for the CaKα peak was 90,900 and that for the Sr_L peak was 1,300. The molar ratio was obtained from these counts, corrected for the relative emission efficiencies of pure CaF_2 and SrF_2 crystal standards. The Ca/Sr ratio is very similar to that obtained by Bidwell *et al.*, (1986) in the single statolith of larval *Aplysia californica* and is close to the molar ratio of sea water (103:1) given by Nicol (1976).

The presence of strontium in the statoconia is of interest in light of the report (Bidwell *et al.*, 1986, Gallager *et al.*, 1988) that the strontium content in the medium in which embryos are reared has a profound effect on the development of both the shell and the statoliths in a number of molluscan species. Roger Hanlon (Marine Biomedical Institute, Galveston, Texas) has found similar results in cephalopods (pers. comm.). We had previously studied the statoliths of individuals of several species of hatchling cephalopods which exhibited a behavioral anomaly, in that they followed a "corkscrew" pattern of swimming or "somersaulted"; *i.e.*, were generally unable to swim in an oriented manner (Colmers *et al.*, 1984). Such individuals were termed "spinners". The only structural abnormalities in these animals were in their statoliths. Hanlon has now found that in two species of octopus (large- and small-egged species), *Sepia* and two species of squid, if there is no strontium in the sea water in which the embryos are raised, all animals are spinners. If the strontium level is raised to 4 ppm, approximately one half of the individuals are spinners and when the strontium is raised to the normal 8 ppm, all animals exhibit normal behavior. This requirement for strontium to obtain normal

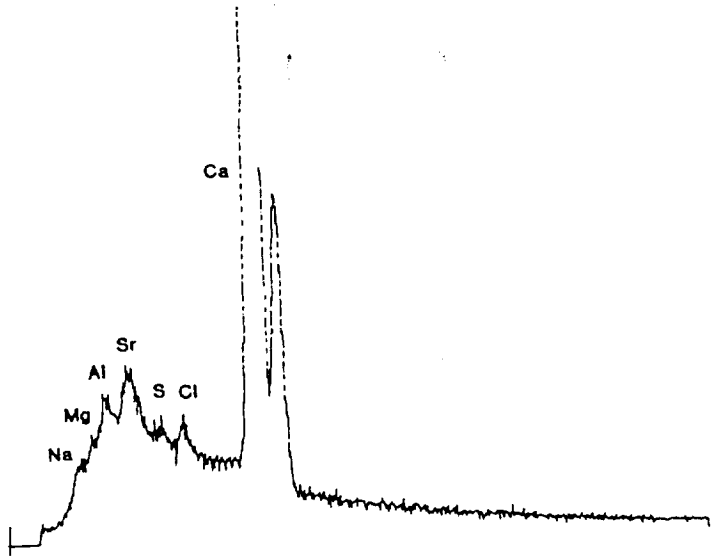

FIGURE 9. Energy-dispersive X-ray microprobe spectrum of analysis of a single deionized-water-rinsed, uncoated statoconium. Note prominent CaKα peak, which is off-scale, and smaller CaKβ peak. Clear peaks for Na, Mg, Al (artifact, see text), Sr, S and Cl are also seen. Full scale = 4096 counts. Mod. from Wiederhold *et al.* (1986).

development of molluscan statoconia would appear to be analogous to the requirement for manganese for normal otoconial development in mammals (Erway *et al.*, 1970; Lim & Erway, 1974).

The molecular basis for the strontium requirement for otoconial development is unknown. Carlstrom (1963) stated that strontium is known to favor the formation of the aragonite form of calcium carbonate. However, aragonite can be formed in the absence of strontium (Carlstrom, 1963; Ross & Pote, 1984; Mann *et al.*, 1983). Whereas the otoconia of all mammals, aves and some reptilia are made of calcite, many fish and some amphibia have otoconia of aragonite (Carlstrom, 1963; Ross & Pote, 1984; Mann *et al.*, 1983). The lizard *Podarcis s. sicula* has calcite otoconia in the lagena, aragonite otoconia in the endolymphatic duct and sac and a mixture of the two in the saccule (Marmo *et al.*, 1981). Lowenstam *et al.* (1984) have recently shown that the statoconia in tetrabranchian cephalopods (*Nautilus*) as well as the statoliths of all of the dibranchian cephalopods (cuttlefish, squid and octopus) are aragonite. The otoconia of the *Nautilus* are about 1% by weight strontium, whereas the statoliths of the other cephalopods are about 0.5% strontium by weight. The shell and other hard parts of *Nautilus* are also made up of aragonite, but their strontium concentration is less than that of the statoconia. Crick *et al.* (1985) have shown that the Ca/Sr ratio is 4.5 times greater in the shell of *Nautilus*, compared to that of sea water. Thus strontium is more effectively excluded from the shell than from the statoconia, even though both are made of aragonite. Aragonite is felt to be advantageous for *Nautilus*, in that it is less brittle than calcite, and thus makes the shell and its chambers better able to withstand the high hydrostatic

pressures at the depths of 500–900 meters where these animals frequently live. The crystal structure of the *Aplysia* statoconia is not known, but the plate structure, particularly evident in Figure 8, suggests that they too are aragonite. This type of formation is typical of aragonite but not of calcite in the lizard (*cf.* Figs. 1, 2 & 3, Marmo *et al.*, 1981). However, Ross & Peacor (1975) illustrated plate-like features on rat otoconia, which are calcite.

The advantage of having statoconia made of aragonite rather than calcite is not obvious. Perhaps in species with independent statoconia there is enough friction as the stones tumble over one another that the physical properties of aragonite would make them less susceptible to "wear" than if they were made of calcite. In mammals and other species in which the otoconia are embedded in a gelatinous membrane, the stones are prevented from striking one another. In some spinner cephalopods which have abnormal statoliths, the crystal structure is grossly different from that of normal animals. Whereas in normal octopus, the statoliths are a concretion of spindle-shaped crystals 2–3μm long (Dilly, 1976; Colmers *et al.*, 1984), the statolith of a spinner *Octopus joubini* illustrated in Colmers *et al.* (1984), is a large mass of tightly packed prismatic crystals. Thus, changes in crystal structure of the developing statoliths in the low-strontium sea water probably contributed to the spinner trait. In those specimens which failed to produce any statoliths, the lack of strontium could have been sufficient to prevent the precipitation of any stable form of $CaCO_3$.

CONCLUSIONS

In light of the profound influence of the trace element strontium on molluscan statoconia formation and of manganese on the formation of mammalian otoconia, it will be of interest to see if future research reveals similar mechanisms responsible for the abnormal crystal structure and shapes of aberrant otoconia seen in normal (Johnsson *et al.*, 1980) or abnormal (Ross & Peacor, 1975) laboratory animals and in cases of human otopathology (Johnsson *et al.*, 1982). If the membranous framework upon which the molluscan statoconia are built is generated within the sensory receptor cells, it would appear likely that the degree of physiological activity in the receptors influences the formation and growth of the statoconia. This would imply that the maintenance of the appropriate number and form of statoconia is a dynamic, rather than passive, process.

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